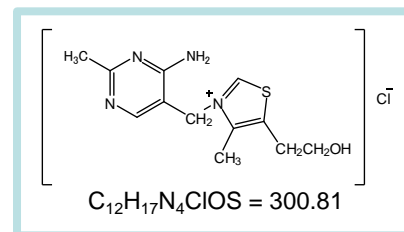


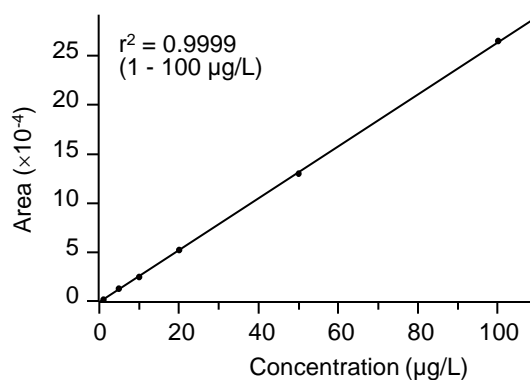
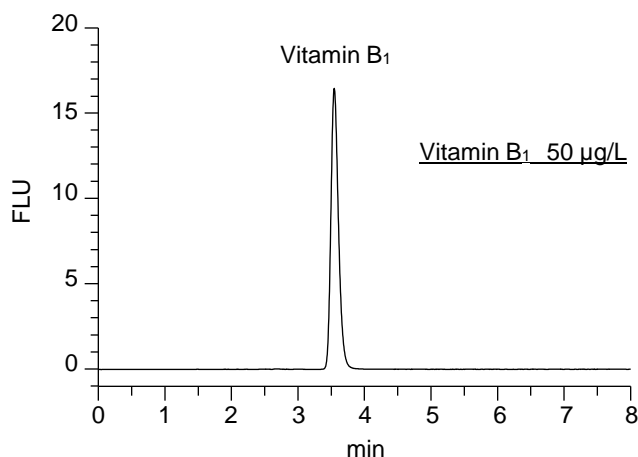
Vitamin B<sub>1</sub> (Thiamine) is a physiologically-active substance classified as a water-soluble vitamin. It naturally exists as thiamine and its phosphate ester form, but is absorbed as thiamine in bodies as the phosphate ester is cleaved. Vitamin B<sub>1</sub> has the effect to change sugars to energy by assisting glycolytic enzymes and it is highly contained in pork, eels, brown rice, etc. The vitamin B<sub>1</sub> deficiency leads to impaired glycolytic energy metabolism, resulting in fatigability and the numbness or swelling of limbs. This time, the analysis was performed by using Chromaster, Hitachi's high performance liquid chromatograph, in accordance with the "High Performance Liquid Chromatograph Method," which is one of the analysis methods specified in the Standard Methods of Analysis in Food Safety Regulation(\*). This is the post-column derivatization method in which vitamin B<sub>1</sub> is converted to thiochrome with alkali potassium ferricyanide for the fluorescence detection.

(\* Standard Methods of Analysis in Food Safety Regulation, Chemistry (Japan Food Hygiene Association, 2005)

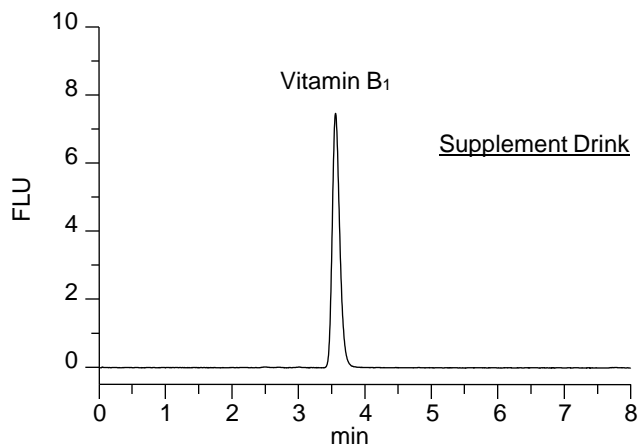


[Structural Formula of Vitamin B<sub>1</sub> (Thiamine)]

■ Analysis Examples of Vitamin B<sub>1</sub> Standard Sample and Supplement Drink



[Linearity for Vitamin B<sub>1</sub>]



<Analytical Conditions>

Column : LaChrom II C18 (5 µm) 4.6 mm I.D. × 150 mm  
 Eluents : Phosphate buffer (pH 2.2) / Methanol = 9/1(v/v)  
 Flow rate : 0.8 mL/min  
 Column temperature : 40 °C  
 Reaction reagent : 0.01 % Potassium ferricyanide • 15 % Sodium hydroxide  
 Reaction reagent flow rate: 0.4 mL/min  
 Reaction temperature : 40 °C  
 Detection wavelength : FL Ex 375 nm, Em 440 nm  
 Injection vol. : 20 µL

<Eluent Preparation Method>

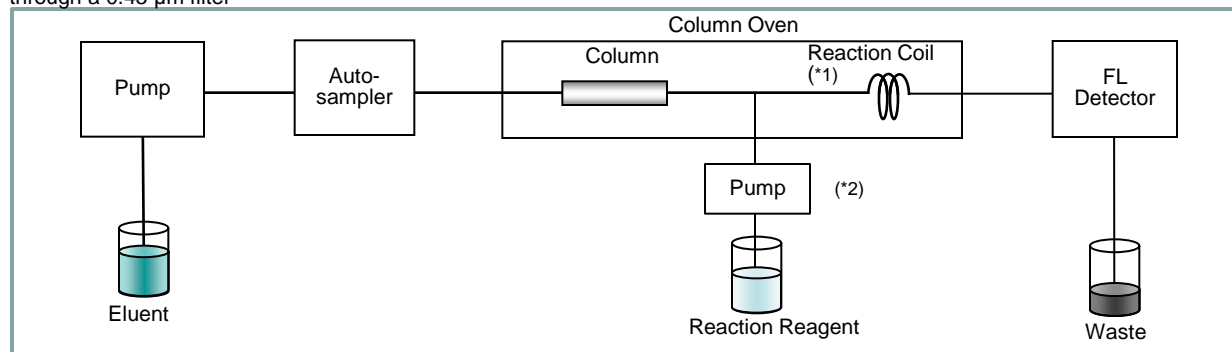
Dissolve 1.2 g of sodium dihydrogen phosphate and 21.1 g of sodium perchlorate monohydrate in water to make 1 L and adjust the pH to 2.2 with perchloric acid. Mix 900 mL of this buffer solution with 100 mL of methanol.

<Reaction Reagent Preparation Method>

Weigh out 50 mg of potassium ferricyanide and make the volume to 500 mL with 15% sodium hydroxide solution. Use a plastic container.

<Sample Preparation Method>

Dilute to 10000 times with 0.1 N hydrochloric acid and filter through a 0.45 µm filter



[Schematic Diagram]

(\*1) 0.8 mm I.D. × 1 m reaction coil is described in the test method. However, 0.25 mm I.D. × 3 m coil was used this time.

(\*2) To prevent a decrease in the sensitivity, the resin tube filter for the reaction pump inlet and resin flow channel filter were used instead of the SUS filters.

Main system configuration: Chromaster 5110 Pump × 2, 5210 Autosampler, 5310 Column Oven, 5440 Fluorescence Detector, Vitamin B<sub>1</sub> Analysis Kit, Column

NOTE: These data are an example of measurement; the individual values cannot be guaranteed.